UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/534,978	11/09/2005	Zhuangwu Li	159976	4719

38598 7590 03/28/2007

ANDREWS KURTH LLP
1350 I STREET, N.W.
SUITE 1100
WASHINGTON, DC 20005

EXAMINER	
THOMAS, DAVID C	
ART UNIT	PAPER NUMBER
1637	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	03/28/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/534,978	LI ET AL.	
	Examiner	Art Unit	
	David C. Thomas	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 January 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-20* is/are pending in the application.

4a) Of the above claim(s) *16-19* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-15 and 20* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *16 November 2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election with traverse of Group 1, claims 1-15 and 20 in the reply filed on January 12, 2007 is acknowledged. Claims 16-19 are withdrawn from further prosecution. The traversal is on the grounds that the X reference, Burger et al. (Eur. J. Cancer (1997) 33:638-644) does not anticipate at least one of the methods claims. This is not found persuasive for several reasons. First, Burger tests samples from untreated and cisplatin-treated cells for comparison of telomerase activity to determine the effectiveness of the treatment and therefore anticipates claim 20. Furthermore, Burger teaches most of the limitations of claims 1 and 16, with the exception that the polymerase is placed in the first reaction mixture instead of the second reaction mixture. Since the step at which the polymerase is added is not critical to the success of the reaction and would be determined by routine optimization of the assay, it is obvious to modify the order of addition of reaction components as needed.

The requirement is still deemed proper and is therefore made FINAL.

Claim Rejections - 35 USC § 102

2. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

3. Claim 20 is rejected under 35 U.S.C. 102(b) as being anticipated by Nakamura et al. (J. Cell. Physiol. (2001) 187:392-401).

With regard to claim 20, Nakamura teaches a method for monitoring the effectiveness of treatment of a subject with an agent that inhibits telomerase activity (inhibitors of telomerase were tested as therapeutic strategy against human liver cancer cells, p. 393, column 1, lines 15-24), said method comprising:

obtaining a pre-administration sample from the subject prior to administration of the agent and detecting a level of telomerase activity in the pre-administration sample (cell lines were obtained from human liver cancers, p. 393, column 1, line 61 to column 2, line 8);

detecting the level of telomerase activity in the pre-administration samples (level of telomerase activity was measured in control samples prior to treatment, p. 393, column 2, lines 18-21; controls without treatment are seen as 0 level in Figure 3 and lane 1 of Figure 4A);

detecting the level of telomerase activity in the post-administration samples (level of telomerase activity was measured in treated samples in both dose- and time-dependent manners, p. 395, column 2, lines 5-17); and

comparing the level of telomerase activity in the pre-administration sample with the level of telomerase activity in the post-administration sample or samples (effect of treatment is seen as decrease of telomerase activity with increasing dose and time of treatment compared to control samples, p. 395, column 2, line 17 to p. 397, column 1, line 7 and Figures 3 and 4B).

Claim Rejections - 35 USC § 103

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

6. Claims 1-6, 8-13 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Harley et al. (U.S. Patent No. 5,891,639) in view of Elmore et al. (Diagn. Mol. Pathol. (2002) 11:177-185).

With regard to claims 1, 5 and 13, Harley teaches a method for detecting and quantifying telomerase activity in a biological sample (for overview, see column 2, line 63 to column 3, line 18), the method comprising the steps of:

adding the biological sample to a reaction tube (cell extract is added to tube containing reaction components, column 28, lines 36-44) comprising:

a first reaction mixture comprising a first primer and nucleoside triphosphates (telomerase primer (TS) and other reaction components including dNTPs are added to tube prior to sample addition, column 28, lines 36-44);

a second reaction mixture comprising a second primer and a DNA polymerase (second primer or polymerase is added under a wax layer, column 11, lines 25-30 and column 28, lines 17-20); and

a wax layer separating the first reaction mixture from the second reaction mixture in the reaction tube (wax layer is used to separate telomerase extension reaction from the amplification reaction, and thus components such as the polymerase or second primer of the amplification reaction are separated the extension reaction components until after the telomerase reaction, column 11, lines 23-30 and column 28, lines 13-17);

incubating the biological sample with the first reaction mixture under conditions suitable for a telomerase to produce an extension product from the first primer, said extension product having a 3' end (telomerase extension reaction is performed for 10 minutes at 20°C to allow TS primer extension, column 28, lines 47-48);

admixing the extension product with the second reaction mixture by melting the wax layer (sample tubes were transferred to a thermocycler to begin cycling, with the wax layer melting upon temperature reaching 70 °C, column 28, lines 47-54);

amplifying the extension product using polymerase chain reaction under conditions that allow the detection of telomerase activity from a single 293T cell (samples are subjected to thermocycling to amplify the extension products, column 28,

lines 50-52; the assay can be sensitive down to a single cell, column 43, lines 44-46 and column 44, lines 15-19), and

quantifying the amplified extension product using a control template (an internal control nucleic acid can be added to the reaction mixture in known amounts and amplified with different primers than used for the telomerase-extended substrate, column 14, lines 45-52).

With regard to claim 2, Harley teaches a method wherein the biological sample is added in the form of a cell or tissue extract (cell extract is added to tube containing reaction components, column 28, lines 36-44 and column 34, lines 1-37).

With regard to claim 3, Harley teaches a method wherein the polymerase chain reaction is detected by using a fluorescently labeled probe oligonucleotide that binds to a sequence between the first and the second primers (products extended by telomerase and amplified can be detected with TaqMan probes which hybridizes to internal site between primers, column 15, lines 26-32 and column 41, lines 45-54).

With regard to claim 4, Harley teaches a method wherein the real-time polymerase chain reaction is performed in the presence of a fluorescent dye that binds preferentially to double-stranded DNA (detection of telomerase-extended substrates or amplification products can be performed with SYBR Green dye, which exhibits enhanced fluorescence when bound to double-stranded nucleic acids, column 21, lines 20-24 and 35-40).

With regard to claim 6, Harley teaches a method further comprising:

elongating the extended product at the 3' end by one of polyadenylation and ligation (extension of telomerase primer can be performed with DNA ligase and can be extended multiple times, column 9, lines 37-52).

With regard to claim 8, Harley teaches a method for detecting and quantifying telomerase activity in a sample cell (detection of telomerase in situ, column 3, lines 23-26), the method comprising the steps of:

introducing into a sample cell a first primer and nucleoside triphosphates (telomerase substrate in form of plasmid containing TS sequence is internalized in cell, column 36, line 56 to column 37, line 7; cells are permeabilized to allow for detection by PCR using dNTPS and Taq polymerase, column 37, lines 42-45 and line 62 to column 38, line 16);

incubating the sample cell under conditions suitable for a telomerase to produce an extension product from the first primer (cells are incubated after internalization of substrate, column 37, lines 29-31);

amplifying the extension product using polymerase chain reaction (PCR condition are established after permeabilization, column 37, line 62 to column 38, line 16); and

quantifying the amplified extension product using a control template (an internal control nucleic acid can be added to the reaction mixture in known amounts and amplified with different primers than used for the telomerase-extended substrate, column 14, lines 45-52).

With regard to claim 9, Harley teaches a method further comprising:

lysing the sample cell with a lysis buffer (after incubation with the internalized substrate, cells are treated with proteases to permeabilize the cells, column 37, lines 55-60).

With regard to claim 10, Harley teaches a method wherein the first primer is introduced into the sample cell (internalization of substrate is achieved using passive internalization, microporation, or electroporation, column 37, lines 20-31).

With regard to claim 11, Harley teaches a method wherein the first primer is introduced into the sample cell by a procedure comprising:

passing the cell through a needle at least once (fine needle aspirates used to biopsy tumor tissue, column 24, lines 26-30); and

culture the cell in a culture medium containing the first primer (substrate is electroporated into cells placed in DMEM media, column 37, lines 26-29).

With regard to claim 12, Harley teaches a method wherein the polymerase chain reaction is performed in the presence of a fluorescent dye that binds preferentially to double-stranded DNA (detection of telomerase-extended substrates or amplification products can be performed with SYBR Green dye, which exhibits enhanced fluorescence when bound to double-stranded nucleic acids, column 21, lines 20-24 and 35-40).

With regard to claim 15, Harley teaches a method for detecting and quantifying telomerase activity in a biological sample, the method comprising the steps of:

adding the biological sample to a reaction tube (cell extract is added to tube containing reaction components, column 28, lines 36-44) comprising:

a first reaction mixture comprising a first primer and nucleoside triphosphates (telomerase primer (TS) and other reaction components including dNTPs are added to tube prior to sample addition, column 28, lines 36-44);

a second reaction mixture comprising a second primer and a DNA polymerase (second primer or polymerase is added under a wax layer, column 11, lines 25-30 and column 28, lines 17-20); and

a wax layer separating the first reaction mixture from the second reaction mixture in the reaction tube (wax layer is used to separate telomerase extension reaction from the amplification reaction, and thus components such as the polymerase or second primer of the amplification reaction are separated the extension reaction components until after the telomerase reaction, column 11, lines 23-30 and column 28, lines 13-17);

incubating the biological sample with the first reaction mixture under conditions suitable for a telomerase to produce an extension product from the first primer, said extension product (telomerase extension reaction is performed for 10 minutes at 20°C to allow TS primer extension, column 28, lines 47-48);

elongating the extended product at a 3' end by one of polyadenylation and ligation (extension of telomerase primer can be performed with DNA ligase and can be extended multiple times, column 9, lines 37-52);

admixing the extension product with the second reaction mixture by melting the wax layer (sample tubes were transferred to a thermocycler to begin cycling, with the wax layer melting upon temperature reaching 70°C, column 28, lines 47-54);

amplifying the extension product using a polymerase chain reaction under conditions that allow the detection of telomerase activity from a single 293T cell (samples are subjected to thermocycling to amplify the extension products, column 28, lines 50-52; the assay can be sensitive down to a single cell, column 43, lines 44-46 and column 44, lines 15-19); and

quantifying the amplified extension product using a control template (an internal control nucleic acid can be added to the reaction mixture in known amounts and amplified with different primers than used for the telomerase-extended substrate, column 14, lines 45-52),

wherein the second primer comprises a nucleotide sequence that is complementary to the nucleotide sequence at a 3' end of the elongated extension product (second primer, the downstream CX primer, is complementary to sequences at end of the telomerase extension product, column 10, lines 18-24).

Harley does not teach a real-time polymerase chain reaction for *in vitro* and *in situ* analysis. Harley also does not teach a second primer that is a single-labeled fluorogenic primer that produces an increased amount of fluorescence emission when the fluorogenic primer is incorporated into double-stranded polymerase chain reaction product. Harley also does teach a method wherein both the polymerase and second primer are separated from the first reaction mixture by a wax layer.

Elmore teaches a method of quantitative analysis of telomerase activity in breast cancer specimens using real-time PCR (p. 179, column 1, lines 2-7), including the use

of a probe labeled with a single fluorogenic compound and a quencher moiety to allow real-time detection (p. 178, column 2, lines 24-27 and Figure 1).

Elmore does not teach methods for detecting and quantifying telomerase activity in a biological sample wherein first and second reaction mixtures are separated by a wax layer and mixed by melting the wax layer. Elmore also does not teach methods for detecting and quantifying telomerase activity in a sample cell by introducing primers and nucleotides into the cell.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to combine the methods of Harley who teaches a method of detecting telomerase activity using conventional end-point PCR and reaction mixes separated by a wax barrier and those of Elmore who teaches a method of detecting telomerase activity using real-time PCR, since the two reaction-mix approach using a wax barrier is easily adapted to a real-time PCR format. Thus, an ordinary practitioner would have been motivated to combine the methods of Harley and Elmore since the development of a fluorescent real-time assay simplifies the overall assay setup, avoids the use of radioactivity, and improves accuracy and turnaround time, which are all important factors in clinical tests (Elmore, p. 179, column 2, lines 1-8 and p. 184, column 2, lines 24-50). Furthermore, the in situ assay can also be performed in real-time using a fluorescent primer (Elmore, Figure 1) that can enter permeabilized cells along with other PCR reagents (Harley, column 37, line 62 to column 38, line 16).

Though Harley does not teach a method of sequestering both a polymerase and second primer in a tube under a wax layer, this reference teaches that either reagent

can be separated from the other mix in this manner (Harley, column 11, lines 25-30). The purpose of the wax barrier is to separate the telomerase extension reaction from the amplification reaction until after the telomerase extension products are formed, and the removal of either the downstream primer or the polymerase will achieve this. The other reagent can be present in the first mixture since amplification will not occur without both the downstream primer and polymerase present. However, it is obvious that both reagents could be placed under the wax layer since there is no functional reason or obvious advantage to have or not have one of the reagents present in the telomerase mixture. To this end, Harley teaches methods to provide any of the reagents either independently or in a mixture as a liquid or in lyophilized form to improve ease of use, with proper conditions in place to stabilize degradable reagent such as the use of stabilizers (Harley, column 26, lines 10-18). Thus, it would be obvious to an ordinary practitioner that one or both reagents could be separated from the first reaction mixture using a wax barrier.

7. Claims 7 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Harley et al. (U.S. Patent No. 5,891,639) in view of Elmore et al. (Diagn. Mol. Pathol. (2002) 11:177-185) and further in view of Choo et al. (GenBank Accession No. AX395585 (2002) and WO 02/04488).

Harley and Elmore together teach the limitations of claims 1-6, 8-13 and 15.

Neither Harley nor Elmore teach a method wherein the control template has a nucleotide sequence recited in SEQ ID NO:2.

With regard to claims 7 and 14, Choo teaches a method wherein the control template has a nucleotide sequence recited in SEQ ID NO:2 (SEQ ID NO:2 is identical to 68-base sequence taught by Choo, TSR8, used as specific control template for telomerase assays, p. 92, line 3 and p. 98, lines 2-4).

Choo does not teach methods of detecting and quantifying telomerase activity in a biological sample using a real-time PCR assay wherein first and second reaction mixtures are separated by a wax layer and mixed by melting the wax layer. Elmore also does not teach methods for detecting and quantifying telomerase activity in a sample cell by introducing primers and nucleotides into the cell.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to combine the methods of Harley who teaches a method of detecting telomerase activity using conventional end-point PCR and reaction mixes separated by a wax barrier and those of Elmore who teaches a method of detecting telomerase activity using real-time PCR, with those of Choo, who teaches a specific substrate that can serve as a positive control template for quantitation of telomerase activity. Thus, an ordinary practitioner would have been motivated to combine the methods of Harley and Elmore for a two-reaction mix fluorescent real-time assay with a control template that allows quantitation of telomerase assays by running a parallel amplification of a similar target sequence containing eight copies of the telomeric repeat. The use of such a control template allows the effects of various conditions and inhibitors of the telomerase extension and amplification reactions to be measured.

Conclusion

8. Claims 1-15 and 20 are rejected. No claims are allowable.

Correspondence

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to David C. Thomas whose telephone number is 571-272-3320 and whose fax number is 571-273-3320. The examiner can normally be reached on 5 days, 9-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

David C. Thomas 3/23/07

David C. Thomas
Patent Examiner
Art Unit 1637

JEFFREY FREDMAN
PRIMARY EXAMINER
3/23/07